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Focal segmental glomerulosclerosis and soluble urokinase-type plasminogen activator receptor

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DESIGNAÇÃO DA ÁREA DO PROJECTO

TÍTULO ~~DISSERTAÇÃO~~/MONOGRAFIA (riscar o que não interessa)

Focal Segmental Glomerulosclerosis and Podocyte Proximal-type Pleomorphism Actuates receptor

ORIENTADOR

Luis Coentrão

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Dedicatória:

Para os meus pais,

Para os meus avós

1. Introduction

Focal segmental glomerulosclerosis (FSGS) is a generic term that can be used to describe a morphological/ histological pattern of injury, as well as the name of a primary glomerular disease [1]. Histologically, it is characterized by sclerosis, hyalinosis, foam cell infiltration, vacuolization of podocytes and podocyte infiltration [2]. Primary FSGS represents approximately 40% of idiopathic nephrotic syndromes. Although idiopatic nephrotic syndrome is a rare disease with an incidence of 7 per 1 million, it often leads to severe renal damage and endstage renal disease (ESRD) [3].

The pathophysiological cause of primary FSGS is still not completely identified [4]. Recently, our knowledge of FSGS has changed dramatically with a major focus on the podocyte as the starting line of the kidney alteration [5]. In fact, podocyte foot process effacement marks the first ultrastructural step related with loss of function in glomerular permeability and the typical proteinuria of FSGS. Despite podocyte genetic defects are a recognized cause of human FSGS, FSGS also appears in the absence of this defects. In some cases, proteinuria may recur after just a few hours or days after kidney transplantation. In fact, almost 30% of adult and pediatric FSGS patients have post-transplant recurrence [6].

These findings, allied to the fact that some patients with FSGS respond to treatment with plasmapheresis, indicate that there may be a circulating factor that changes the glomerular permeability function [7].

The aim of this review was to study the literature that relates urine and/or serum suPAR levels with primary FSGS and post-transplant recurrence in patients with FSGS.

2. Focal segmental glomerulosclerosis:

2.1. Pathogenesis

The major feature of FSGS is proteinuria. Indeed, the nephrotic syndrome appears at the presentation in about 90% of children and 60% of adults with focal segmental glomerulosclerosis. The pathogenesis of focal segmental glomerulosclerosis can be divided in two categories: primary and secondary. In both cases, the loss of integrity of the glomerular filtration barrier in the glomeruli of the kidney cortex can lead to nephrotic proteinuria. This barrier is divided by three layers: the fenestrated endothelium, the glomerular basement membrane and the podocytes.

Podocytes are extremely differentiated and polarized epithelial cells, which integrity is given by a central actin cytoskeleton core. In the outer aspect of the glomerular wall, they have foot process, linked to each other by a slit diaphragm, composed essentially by nephrin. These cells cannot repair by cell division, making podocyte

depletion a major mediator of glomerulosclerosis [8]. Therefore, changes in podocytes morphology and function can lead to FSGS. It starts by retraction of the foot processes and extension of the connecting plasma membrane between two feet, which leads to podocyte foot process effacement. This can lead to podocytes apoptosis and necrosis and develop to loss of glomerulus with scar formation and eventual loss of the entire nephron. [5]

2.2. Morphologic variants:

FSGS has an evident diversity of morphologic lesions, that can differ in their location to the vascular and tubular pole, as well as the glomerular hipercellularity and capillary collapse [9]. The Columbia Classification is a histologic classification that recognizes 5 types: perihilar, cellular, tip, collapsing and not-otherwisespecified, which can be applied to primary and secondary forms of FSGS:

- The cellular variant is featured by a segmental expansible endocapillary hypercellularity obliterating capillary lumen, generally with severe footprocess effacement. This type is usually associated with primary FSGS and is the least common variant.
- The collapsing variant is defined as a segmental or global implosive collapse of glomerular capillaries with hypertrophy and hyperplasia of the overlying visceral epithelial cells, sometimes resembling a "pseudo-crescent". This variant has the worst prognostic, with poor rate of response to glucocorticoids and a fast course to renal failure.
- Tip lesions are associated to a single segmental lesion involving the tubular pole and are often associated with primary FSGS. It usually presents with abrupt onset of the nephrotic syndrome and has the best prognosis, with highest responsivity to glucocorticoids and the lowest risk of progression.
- The perihilar variant is characterized by perihilar hyalinosis and sclerosis at the glomerular vascular pole in the majority of glomeruli with segmental lesions. It is more common in adaptive FSGS and is related to obesity, reflux nephropathy, hypertensive nephrosclerosis, sickle cell anemia and renal agenesis.
- Cases that don't meet the criteria for the other 4 variants are classified as FSGS not otherwise specified (NOS). This is the usual generic form of FSGS. The defining lesion of NOS variant is a segmental obliteration of the glomerular capillaries by extracellular matrix. Hyalinosis, endocapillary foam cells, capsular adhesion and parietal cell coverage of the sclerotic lesions can also be present. Several studies suggest this is the most common variant. Other variants can evolve into NOS FSGS over time. [10]

2.3. Secondary FSGS:

The secondary causes of FSGS can be divided in four sub-types: Familial or genetic, virus-associated, drug-induced and adaptive form.

Genetic forms of FSGS are due to gene mutations, essentially in nephrin and podocin, components of the slit diaphragm [11]. Most mutations have an autosomal recessive transmission, which manifests earlier in life. However, autosomal dominant transmission (e.g., mutations in genes encoding α-actinin-4 and transient receptor potentional cation channel 6) usually presents later. This genetic defects were found in approximately 65% of patients with FSGS who presented this histological pattern in the first year of life [12].

Viruses can affect the podocyte by direct infection or by the release of inflammatory mediators, like cytokines. Human immunodeficiency virus type 1 (HIV-1) is the most studied virus, which directly infects podocytes and tubular epithelial cells [13]. Parvovirus B19 is another well-known virus that can infect podocytes and tubular cells, leading to a collapsing glomerulopathy [14]. Simian virus 40, cytomegalovirus and Epstein-Barr virus are also associated with renal lesion and FSGS.

Drug-induced FSGS can be associated to heroin abuse [15]. However, FSGS is also linked to other drugs, like bisphosphonate pamidronate, an osteoclast inhibitor [16], all forms of interferon (alfa, beta and gamma) [17] and mTOR (also known as Rapamycin) [18].

At last, adaptive FSGS is a secondary form that results from structural and functional adaptations, mediated by intrarenal vasodilatation, increased glomerular capillary pressures and plasma flow rates [19].

2.4. Primary FSGS:

Primary FSGS has been related to a supposed circulating permeability factor, which is suggested by some indirect evidence: the skill to regulate proteinuria by immunoadsorption, potential disease recurrence minutes after transplantation, therapeutic reduction of proteinuria after plasmapheresis and the induction of proteinuria in experimental animals by infusion of patient plasma or its fractions.

In fact, soluble urokinase-type plasminogen activator factor receptor (suPAR), is strongly being suggested as a major candidate. Serum concentrations levels of suPAR are higher in patients with FSGS. However, this is not observed in patients with minimal change disease (MCD) or membranous nephropathy (MN). Elevated serum levels of suPAR (cut-off of 3000 pg/mL) were found in up to 66% of patients with FSGS [20].

2.5. Treatment:

Therapy goal is to induce a complete or partial remission of proteinuria and to maintain renal function.

Pediatric patients with nephrotic syndrome are treated empirically with Prednisone (60 mg/m^2 per day) for 4-6 weeks.

Adults usually need a renal biopsy before therapy. Once the diagnosis is made, potential secondary causes for nephrotic syndrome must be ruled out. Initial therapy of patients with FSGS is RAS blockade and dietary sodium restriction. High dose glucocorticoid therapy can be given as 1 mg/ kG of body weight or as 2 mg/kG on alternate days. In children, this treatment has the duration of 4-6 weeks and in adults of 16 weeks [21]

In cases of glucocorticoids resistant FSGS and patients with diabetes, psychiatric disorder and severe osteoporosis (in which therapy with glucocorticoids has severe side effects) the therapy is based in a calcineurin inhibitor, like cyclosporine [22].

The imunossupressive action of these agents may only have a small role in podocyte and glomerular repair. Instead, they have direct effects on podocytes through the regulation of survival, maturation and stability pathways. They also are able to regulate the expression and distribution of components of the slit diaphragm and the cytoskeleton [23]. Additionally, Rituximab has also shown good results in the therapy of FSGS. However, this agent may not be effective in steroidresistant FSGS.

Plasmapheresis and immunoadsorption may be considered in patients who don't respond to severe immunossupressive treatment [24].

Approximately 40% of patients with primary FSGS and ESRD who undergo a kidney transplant, have recurrence of FSGS. There are many risk factors for this event, including: younger patients, non-black race, rapid course to ESRD, substantial proteinuria in the period before transplant and previous allografts to recurrence.

In these patients, plasmapheresis can be used at the beginning of recurrence [25].

3. The Soluble Urokinase Plasminogen Activator Receptor

3.1. Structure and function:

The urokinase-type plasminogen activator receptor (uPAR) is a glycosylphosphatidylinositol (GPI)-linked membrane protein. It contains three domains and is present on numerous immunologically active cells like monocytes, activated T-lymphocytes and macrophages, but also in endothelial cells,

keratinocytes, fibroblasts, smooth muscle cells, megakaryocytes and some neoplasic cells [26]. uPAR is formed by approximately 90 amino-acids and has three homologous domains (DI, DII and DIII) [27]. These domains are encoded by separate exon groups of the *Plaur* gene [28].

uPAR can bind to many ligands like uPA, Vitronectin, and integrins. By binding to its receptor (uPAR), uPA can mediate many cellular functions like adhesion, migration, differentiation and proliferation [29].

Components of full-length uPAR and its ligands interact with integrin co-receptors for intracellular signal transduction. uPA also cleaves uPAR in the linker region between D1 and D2, producing a soluble D1 fragment and a membrane-associated D2-D3 fragment. uPAR cleavage prevents uPA binding, which can inactivate the function of uPAR in proteolysis and also the signaling functions of uPAR [30]. Thereby, after the cleavage of the GPI anchor, uPAR is released from the plasma membrane as a soluble molecule (suPAR). Its size ranges from 20 to 50 kDa and it is found in low concentrations in human fluids under physiologic conditions [5]. Both the circulating and membrane-bound forms are directly involved in the regulation of cell adhesion and migration through binding of integrins [31]. Indeed, both intact and cleaved suPAR variants can have diagnostic and prognostic values in cancer, inflammatory and metabolic diseases. [27]

Podocyte foot process has an actin cytoskeleton, which is connected to the glomerular basal membrane by $\alpha 3\beta 1$, $\alpha v\beta 3$ integrin and α and β-dystroglycans. Indeed, induction of uPAR intracellular signaling in podocytes can lead to foot process effacement and proteinuria by lipid-dependant activation of $\alpha v\beta 3$ integrin. Therefore, the blockage of $\alpha v\beta 3$ integrin can reduce podocyte motility and had lowered proteinuria in mice [29].

Increased activation of the immune system can lead to higher levels of serum suPAR, which is also recognized in several conditions like human immunedeficiency virus type 1(HIV-1)-infection, malaria, pneumococcal and streptococcus pneumonia, sepsis, bacterial and viral CNS infection, active tuberculosis, and also in various forms of solid tumors [30].

3.2. suPAR and a role in FSGS:

Recent studies describe suPAR as a primary candidate circulating factor in patients with primary FSGS. This studies show that total suPAR levels in serum and urine are elevated in patients with FSGS, and high serum levels may be associated with recurrence in transplanted kidneys [4]. In fact, suPAR can be used to differenciate primary FSGS from other causes of kidney disease and serum and urine suPAR can have a role in risk stratifying kidney transplant candidates with FSGS or patients with native kidney disease undiagnosed [32].

Wei et al stratified FSGS cases in three sub-populations: primary FSGS, recurrent FSGS after transplantation and FSGS without recurrence after transplantation. They verified that the highest concentrations of suPAR were found in the pretransplantation blood of subjects with FSGS who later developed recurrent FSGS after transplantation. It was proposed that pre-transplantation suPAR serum concentration may be a predictor of increased risk of recurrence FSGS after transplantation. They also concluded that suPAR serum concentrations were significantly higher 1 year after transplantation in individuals that developed recurrent FSGS than in FSGS patients who received kidney transplants and then had normal renal functions. Additionally, although suPAR concentrations correlated with the presence of proteinuria, this was not observed with its degree.

3.3. Podocyte foot effacement and uPAR expression:

Wei et al reported increased *Plaur* mRNA in glomeruli of patients with FSGS. Indeed, mice lacking uPAR (Plaur-/-) were protected from lipopolysaccharide (LPS)-mediated induced proteinuria. However, they still developed disease after the expression of a constitutively active β3-integrin. This indicates an association between the development of podocyte foot effacement and uPAR expression [33].

3.4. A role for β-3 integrin:

These investigators searched for the presence of active β3-integrin in podocytes, using the AP5 antibody, which recognizes an N-terminal epitope of β3-integrin that is only available when the integrin is activated. They found that LPS treatment of wild-type mice was linked with a strong induction of podocyte AP5 labeling. This induction was not observed in LPS-treated *Plaur* -/- mice [28].

3.5. Circulating and membrane-bound podocyte uPAR in FSGS:

Other studies addressed if only membrane-bound podocyte uPAR and not circulating suPAR could be associated with the pathogenesis of proteinuria and FSGS. High-dose recombinant mouse $suPAR_{I-III}$ induced proteinuria in *Plaur* knockout mice. In fact, a kidney from these mouse that was transplanted in a wildtype mouse developed proteinuria after LPS-induced suPAR production. These facts suggests that circulating suPAR is independent of uPAR in the activation of β3-integrin [6].

3.6. Clinical results supporting a role of suPAR in FSGS:

Other studies were made to investigate the role of suPAR in human FSGS. It was observed an increased activity of β3-integrin when podocytes were exposed to serum of patients with recurrent FSGS. Alternatively, when serum from patients with complete proteinuria remission after plasmapheresis was used, podocyte β3integrin activity was reduced. It was also demonstrated that this activity was reduced when it was blocked by antibodies against uPAR and β3-integrin inhibitors [34].

Some other clinical data also support these observations. In fact. Wei et al, identified high serum suPAR levels in patients with primary FSGS, but not in the control group. They also described that pre-transplant elevated serum suPAR levels increased risk for recurrence FSGS after transplant [6].

In other study, Wei et al compared serum suPAR concentrations in transplanted FSGS patients 1 year after transplant. They found significantly higher suPAR serum levels in patients that developed recurrent FSGS , compared to those FSGS patients who had normal kidney disease after transplant. They also concluded that serum suPAR levels correlated with the presence, but not the degree of proteinuria.

To analyze if suPAR levels could be decreased by plasmapheresis, Wei et al analysed serum from subjects with recurrent FSGS before and after a single treatment of plasmapheresis and measured suPAR concentrations. They observed that serum suPAR levels were significantly lower. They also tested if plasmapheresis could lower podocyte β3 integrin activity by measuring AP5 signal. Indeed, they concluded that plasmapheresis could significantly lower podocyte β3 integrin activity caused by incubation of podocytes with serum of FSGS patients
[33].

Additionally, it was described that urinary suPAR levels of patients with FSGS were significantly higher compared to those with other glomerular diseases and normal subjects, and positively associated with 24-hour urinary protein excretion in primary FSGS [29].

All these investigations are essential to the supposition that suPAR is the fundamental circulating factor involved in FSGS.

3.7. Clinical results not supporting a role of suPAR in FSGS:

However, some data don't support this correlation. Indeed, some studies suggests that suPAR concentrations are also elevated in minimal change disease (MCD), membranous nephrophaty, IgA nephrophaty, lupus nephritis or non-glomerular chronic kidney disease (CKD). This suggests that suPAR is also involved in the pathogenesis of other glomerular diseases [29]. Other studies found no evidence that pre-transplant serum suPAR was different in recurrent FSGS vs non-recurrent

FSGS, after kidney transplant [32]. Additionally, it was also reported that serum suPAR levels were similar before and after the induction of remission [34]. suPAR levels are also associated to other illness that are not associated primarily with proteinuria, like infections and tumors. Furthermore, ESRD patients may accumulate suPAR, which contributes to elevated suPAR levels [5].

Some FSGS patients without elevated suPAR levels still develop FSGS and recurrent FSGS [6].

4. Conclusion

Primary FSGS pathogenesis has been associated with a permeability factor for several years. suPAR is one of the most probable candidates to corroborate this association. Recent studies suggested that serum and urine suPAR levels are elevated in patients with primary FSGS and after kidney transplant. Wei et al performed a variety of studies that suggests suPAR as a primary mediator of podocyte foot effacement. They concluded that both membrane-bound uPAR and circulating suPAR can activate β-3 integrin. Other studies suggested that podocyte β3-integrin activity and serum suPAR levels were lower after treatments with Plasmapheresis.

More studies are required no confirm if high serum and/or urine suPAR levels are specific of FSGS or are shared with other glomerular diseases. Additionally, it should be verified if high suPAR levels in FSGS patients with ESRD is due to the accumulation of suPAR.

5. Bibliography

[1]- Angioi A, Pani A. FSGS: from pathogenesis to the histological lesion. Journal of Nephrology, 2016;29:517-23

[2]- Han MH, Kim YJ. Pratical application of Columbia classification for focal segmental glomerulosclerosis. BioMed Research International ;2016:9375753

[3]- Königshausen E and LorenzSellin. Circulating permeability factors in primary focal segmental glomerulosclerosis: A Review of Proposed Candidates. BioMed Research International;2016:3765608

[4]- Staeck O, Slowinski T,Lieker I, Wu K, Rudolph B, Schmidt D, Brakemeier S, Neumayer HH, Wei C, Reiser J, Budde K,Halleck F, Khadzhynov D. Recurrent primary focal segmental glomerulosclerosis managed with intensified plasma exchange and

concomitant monitoring of soluble urokinase-type plasminogen activator receptor–mediated podocyte β3-integrin activation. Transplantation 2015 ; 99: 2593–2597

[5]- Reiser J, Nast CC, Alachkar N. Permeability factors in focal and segmental glomerulosclerosis. Advanced Chronic Kidney Disease. 2014; 21: 417–421.

[6]- Wei C et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nature Medicine 2011; 31;17:952-60

[7]-Savin VJ, Sharma R,Sharma M,McCarthy ET, Swan SK, Ellis E,Lovell H, Warady B, Gunwar S, Chonko AM, Artero M, and Vincenti F. Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. The New England Journal of Medicine 1996 4;334:878-83

[8]- D'Agati Vivette D, Kaskel Frederick J, Falk Ronald J. Medical progress: Focal segmental glomerulosclerosis. The New England Journal of Medicine 2011; 365:2398-411

[9]- D'Agati VD, Fogo AB, Bruijin JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. American Journal of Kidney Disease 2004;43:368-82

[10]- Stokes MB, D'Agati VD. Morphologic variants of focal segmental glomerulosclerosis and their significance. Advances in Chronic Kidney Disease 2014;21:400-7

[11]- Tryggvason K, Patrakka J, Wartiovaara J. Hereditary proteinuria syndromes and mechanisms of proteinuria. New England Journal of Medicine 2006;354:1387-401

[12]- Hinkes BG, Musha B, Vlangos CN, et al. Nephrotic syndrome in the first year of life: two thirds of cases are caused by mutations in 4 genes (NPHS1, NPHS2, WT1 and LAMB2). Pediatrics 2007;119:e907-e919

[13]- Bruggeman LA, Ross MD, Tanji N, et al. Renal epithelium is a previously unrecognized site of HIV-1 infection. Journal of the American Society of Nephrology 2000;11:2079-87

[14]- Moudgil A, Nast CC, Bagga A, et al. Association of parvovirus B19 infection with idiopatic collapsing glomerulopathy. Kidney International 2001;59:2126-33

[15]- Friedman EA, Tao TK. Disapperarence of uremia due to heroin-associated nephropathy. American Journal of Kidney Disease 1995;25:689-93

[16]- Markowitz GS, Appel GB, Fine PL, et al. Collapsing focal segmental glomerulosclerosis following treatment with high dose pamidronate. Journal of the American Society of Nephrology 2001;12:1164-72

[17]- Markowitz GS, Nasr SH, Stokes MB, D'Agati VD. Treatment with IFN-α, -β or – λ is associated with collapsing focal segmental glomerulosclerosis. Clinical Journal of the American Society of Nephrology 2010;5:1353

[18]- Vollenbröker B, George B, Wolfgart M, Saleem MA, Pavenstädt H, Weide T. mTOR regulates expression of slit diaphragm proteins and cytoskeleton structure in podocytes. American Journal of Physiology. Renal Physiology 2009;296:F418F426

[19]- Gipson DS, Trachtman H, Kaskel FJ, et al. Clinical trial of focal segmental glomerulosclerosis in children and young adults. Kidney International. 2011; 80:868-878

[20]- Maas RJ, Deegens JK, Wetzels JF. Permeability factors in idiopathic nephrotic syndrome: historical perspectives and lessons for the future. Nephrol Dial Transplant. 2014;29:2207-16

[21]- Banfi G, Moriggi M, Sabadini E, Fellin G, D'Amico G, Ponticelli C. The impact of prolonged immunosuppression on the outcome of idiopathic focal-segmental glomerulosclerosis with nephrotic syndrome in adults. A collaborative retrospective study. Clinical Nephrology. 1991;36:53-9.

[22]- Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, Maxwell DR, Kunis CL. A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. Kidney International. 1999 56:2220-6.

[23]- Schönenberger E, Ehrich JH, Haller H, Schiffer M. The podocyte as a direct target of immunosuppressive agents. Nephrol Dial Transplant. 2011;26:18-24. [24]- Beer A, Mayer G, Kronbichler A. Treatment strategies of adult primary focal segmental glomerulosclerosis: A systematic review focusing on the last two decades. Biomed Research International. 2016;2016:4192578

[25]- Vinai M, Waber P, Seikaly MG. Recurrence of focal segmental glomerulosclerosis in renal allograft: an in-depth review. Pediatric Transplantation. 2010;14:314-25

[26]- T Maria, M Betina and EO Jesper. suPAR: The molecular crystal ball. Disease Markers 27 (2009) 157–172

[27]- Chen JS, Chang LC, Wu CZ, Tseng TZ, Lin JA, Lin YF, and Cheng CW. Significance of the urokinase-type plasminogen activator and its receptor in the progression of focal segmental glomerulosclerosis in clinical and mouse models. Journal of Biomedical Science (2016) 23:24

[28]- Wei C, ller CCM, Altintas MM, Li J, Schwarz K, Zacchigna S, Xie L, Henger A, Schmid H, Rastaldi MP, Cowan P, Kretzler M, Parrilla R, Bendayan M, Gupta V, Nikolic

B, Kalluri R, Carmeliet P, Mundel P & Reiser J. Modification of kidney barrier function by the urokinase receptor. Nature Medicine. Jan;14(1):55-63

[29]- Kronbichler A, Saleem MA, Meijers B, and Shin JI. Soluble urokinase receptors in focal segmental glomerulosclerosis: A review on the scientific point of view.

[30]- Smith HW and Marshall CJ. Regulation of cell signalling by uPAR. Nature Reviews. Molecular cell Biology. 2010 Jan;11(1):23-36

[31]- Hayek SS, Sever S, Ko YA, Trachtman H, Awad M, Wadhwani S, , Altintas MM, Wei C, Hotton AL, French AL, Sperling LS, Lerakis S, Quyyumi AA, and Reiser J. Soluble urokinase receptor and chronic kidney disease. New England Journal of Medicine. 2015 November 12; 373(20): 1916–1925

[32]- Carlos R Franco Palacios, John C Lieske, Hani M Wadei, Andrew D Rule, Fernando C. Fervenza, Nikolay Voskoboev, Vesna D Garovic, Ladan Zand, Mark D Stegall, Fernando G Cosio, and Hatem Amer. Urine but not serum soluble urokinase receptor (suPAR) may identify cases of recurrent FSGS in kidney transplant candidates. Transplantation. 2013 August 27; 96(4): 394–399.

[33]- Wei et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nature Medicine. 2011 Jul 31;17(8):952-60

[34]- Maas RJH, Deegens JKJ and Wetzels JFM. Serum suPAR in patients with FSGS: trash or treasure? Pediatric Nephrology (2013) 28:1041–1048

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